ISOLATION AND GENETIC CHARACTERIZATION OF AMPHIBIAN CHYTRID STRAINS IN CENTRAL TEXAS

by

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DEDICATION

I dedicate this thesis to my family for their constant love and support.

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ABSTRACT

Chytridiomycosis, an emerging infectious disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is a major contributor to declines in amphibian populations worldwide. *Bd* was first described in the 1990s, and there is still much to learn about its regional diversity and origin. The Global Panzootic Lineage (*Bd*-GPL) has been responsible for devastating amphibian population declines and extinctions in Central and South America, Australia, and the western U.S. On the other hand, a few localized endemic lineages have been discovered in regions such as Brazil and Asia, which are areas that have not experienced such severe disease outbreaks. There are still several geographic sampling gaps in the analysis of the global distribution of *Bd*, and relatively few studies have focused on regions in which *Bd* exhibits low virulence, thus creating a bias in our current knowledge of the pathogen's diversity. One such region that has not seen disease-associated declines is the state of Texas. This pathogen has been detected from amphibians in the state, although strains had not been characterized genetically prior to this study. Here, we isolated, cultured, and genotyped strains of *Bd* in Central Texas and compared them to a panel of previously genotyped strains distributed across the globe. Our results support the hypothesis that *Bd* is an introduced pathogen in the region. We found a diversity of *Bd* genotypes yet did not detect geographically based genetic structure in Texas and across North America. Strains in Central Texas are genetically similar to those in the western U.S. that have caused amphibian population

declines, which raises questions about the roles that climate and host resistance play in shaping *Bd*-amphibian disease dynamics in North America.

I. INTRODUCTION

Emerging infectious diseases (EIDs) — those that have recently increased in incidence, impact, virulence, geographic or host range, or have recently evolved — are a growing threat to human health and global biodiversity (Daszak *et al.* 2000; Daszak et al. 2003). An understanding of the evolutionary processes that shape interactions between a pathogen, its hosts, and the environment can provide insights into the origin of an emerging infectious disease, its rate of spread, and potential impact on host populations (Vander Wal et al. 2014). Two different hypotheses can be invoked to explain the origin of an emerging infectious disease; each one has distinct implications for conservation strategies and focuses on different aspects of the host-pathogen-environment epidemiological triangle (Rachowicz et al. 2005). The novel, or emerging, pathogen hypothesis attributes disease outbreaks to the recent spread and exposure of a pathogen to naïve hosts (Alford 2001), thus focusing on the host-pathogen aspect of the disease triangle. The endemic pathogen hypothesis concentrates on the environmental effects on a host and (or) pathogen, attributing disease outbreaks to environmental changes that have altered a previously benign host-microbe relationship (Rachowicz et al. 2005). Each hypothesis carries with it a unique set of testable predictions that relate to the evolutionary history of the pathogen and the degree of coevolution with its host species (Morgan et al. 2007; James et al. 2009; Rosenblum et al. 2013).

Under the novel pathogen hypothesis, a founder effect is expected to occur, as a subset of pathogen genotypes are introduced to a new region followed by rapid spread (James et al. 2009). Evidence for this effect should include low allelic diversity among strains in regions of disease outbreak (James et al. 2009; Velo-Anton et al. 2012), as well

as minimal phylogeographic structure across invaded areas (Morgan et al. 2007; Rosenblum et al. 2013). A moving invasion front is expected under this hypothesis, as a pathogen progressively infects new hosts, which may include multiple species that are equally naïve to the invading disease agent (Laurance et al. 1996). High mortality rates are predicted since hosts have not had sufficient time to evolve defenses to the pathogen (Morgan et al. 2007). It has also been suggested that higher virulence should be selected for on the invasion front, as the pathogen can rapidly cycle through new susceptible and high density host populations (Phillips & Puschendorf 2013). Thus, disease outbreaks may become more devastating as the pathogen spreads through a region. Finally, the novel pathogen hypothesis requires that the infecting agent either has high dispersal ability or can be easily spread through a vector (Morgan et al. 2007).

Under the endemic pathogen hypothesis, the pathogen is believed to have benignly coexisted with its host species for a prolonged period of time until environmental changes altered this relationship (Rachowicz et al. 2005). Possible environmental disruptions include climate change, chemical changes in the environment, and increased host density, all of which are stressors that can have immunosuppressive effects on the host species (Rachowicz et al. 2005). Under certain conditions, these changes may also be conducive to increased pathogen reproduction and transmission (Rachowicz et al. 2005). In contrast to the novel pathogen hypothesis, predictions under the endemic pathogen hypothesis include host specificity, reflecting a period of coevolution and specialization between pathogen and host (Morgan et al. 2007), and phylogeographic structure indicating the evolution of genetically distinct pathogen populations in different regions (Morgan et al. 2007; Rosenblum et al. 2013). One might

also expect to see differential pathogen virulence corresponding to differences in environmental conditions, such as temperature, level of habitat disturbance, and host population density (Rachowicz et al. 2005). Museum specimens that document the presence of a pathogen in a host species in a region prior to a period of disease outbreaks also provide support for the endemic pathogen hypothesis (Rodriguez et al. 2014).

Fungal diseases in particular have been a growing concern over the last several decades and include white-nosed syndrome in bats, snake fungal disease, colony collapse disorder in bees (attributed to the microsporidian *Nosema* sp.), sea-fan aspergillosis in coral, and a variety of plant and crop diseases (Fisher et al. 2012; Fisher et al. 2016). None of these diseases have been as devastating as chytridiomycosis, an epidermal infection in amphibians that is caused by the aquatic chytrid *Batrachochytrium dendrobatidis* (*Bd*) (Lips 2016). A member of the Rhizophydiales order of fungi (Letcher et al. 2006), *Bd* causes infections when its flagellated zoospores encyst in a host's skin and develop into zoosporangia (Longcore et al. 1999). *Bd* was first described and attributed to disease outbreaks in the late 1990s (Longcore et al. 1999) and is now believed to be responsible for declines in amphibian populations dating back at least to the 1970s (Lips et al. 2004). It is found on all continents except Antarctica, is known to have infected over 700 species of amphibians, and has caused mass mortality events and species extinctions in regions of disease outbreak (Lips 2016), which have included Central and northern South America, the western U.S., Australia, and Spain (Lips et al. 2006; Vredenburg et al. 2010; Berger et al. 1998; Bosch et al. 2001). However, certain species and geographic regions have seemingly been unaffected by chytridiomycosis, even where *Bd* is present (Lips 2016).

In an effort to understand the origins and evolutionary history of *Bd*, as well as factors contributing to its differential virulence across host species and regions, sampling and genotyping efforts have been conducted across the globe. In support of the novel pathogen hypothesis, multiple population genetics studies of *Bd* isolates have found little evidence of population structure across different geographic locations (Morehouse et al. 2003; Morgan et al. 2007; James et al. 2009). Low allelic diversity in global isolates of *Bd* was cited by James et al. (2009) as evidence of a recent population bottleneck, followed by multiple introductions around the world. In the Sierra Nevada of California, Morgan et al. (2007) found no evidence of coevolution between *Bd* and two endemic, isolated ranid species, and argued that population structure of the fungal pathogen in the region was indicative of two separate anthropogenic introductions. Lips et al. (2008) provided evidence of multiple introductions of *Bd* in South and Central America followed by rapid spread. Lag times between *Bd* arrival in this region and subsequent amphibian mortalities have decreased, indicating the evolution of increased virulence as the pathogen has invaded the Neotropic zone (Phillips & Puschendorf 2013). The observed spikes in amphibian mortalities across the globe are also indicative of an introduced pathogen. It has been demonstrated that amphibians can evolve tolerance to *Bd* (Savage & Zamudio 2016). Thus, amphibian populations that have a long history of sympatry with *Bd* should not exhibit the dramatic losses that have been observed in recent chytridiomycosis outbreaks. Potential causes of *Bd* introduction around the world include the global pet trade, the spread of the invasive bullfrog, *Rana catesbeiana*, which is cultivated for food in Asia and South America, and the invasive African clawed frog,

Xenopus laevis, which was once exported for use in pregnancy tests (Weldon et al. 2004; Schloegel et al. 2012).

While several lines of evidence corroborate the novel pathogen hypothesis, there is support for endemism as well. Further sampling of *Bd* isolates and whole genome analyses have created a more complex picture of the pathogen's evolutionary history. Phylogenomic analyses of *Bd* have revealed multiple lineages and a much older history than previously realized (Farrer et al., 2011; Rosenblum et al. 2013). These lineages include the widespread global panzootic lineage (GPL), which is implicated in most disease outbreaks and can be further divided into a temperate clade (GPL-1) and a tropical clade (GPL-2), and the putatively endemic and less virulent *Bd*-Cape (found in South Africa and Spain), *Bd*-CH from Switzerland (Farrer et al. 2011), and *Bd*-Korea (Bataille et al. 2013). Recent sampling efforts have led to the discovery of a strain in Japan that may be host specific (Goka et al. 2009) and *Bd*-Brazil (Schloegel et al. 2012; Rodriguez et al. 2014; Jenkinson et al. 2016), which genomic analyses have indicated is the basal lineage in the *Bd* clade (Rosenblum et al. 2013). The discovery of multiple endemic lineages, which are beginning to gain recognition as distinct species (Lips 2016), and a history that dates back possibly over 100,000 years (Rosenblum et al. 2013) provide support for the endemic pathogen hypothesis. Evidence that climate change (Pounds et al. 2006) and habitat disturbance (Becker et al. 2016) can contribute to disease outbreaks or increased host infection loads in certain environments offers insight into how a benign chytrid-amphibian relationship could have evolved into a lethal interaction. However, major disease outbreaks have yet to be attributed to any other *Bd* lineage besides the GPL (James et al. 2015), which is acting as a novel invasive species in many

parts of the world (Rosenblum et al. 2013). In this sense, both the novel and endemic pathogen hypotheses are supported. It appears likely that *Bd* is, in fact, a species complex with multiple taxa endemic to different regions, and that a relatively young lineage, or species (the GPL), which has potential for high virulence in certain environments and when exposed to naïve hosts, has recently spread globally, resulting in the observed disease outbreaks.

Fungal diversity is still poorly understood, and fungal pathogens are particularly notable for having a high degree of cryptic genetic diversity (Fisher et al. 2016). The diversity that has recently been revealed within the *Bd* clade, coupled with the fact that there are still several geographic gaps in our current knowledge of *Bd* genotypes (James et al. 2015), suggests the likelihood of additional lineages yet to be discovered. Relatively few studies have examined *Bd* genotypes from regions in which population declines have not occurred, thus creating a bias in our knowledge of the pathogen's genetic diversity (Bataille et al. 2013). It is likely that *Bd*'s origin can be traced to a region in which amphibians have co-evolved with the fungus and where severe population declines and extinctions are not occurring (James et al. 2015). While recent work has begun to explore such regions of potential origin (Bataille et al. 2013; Jenkinson et al. 2016), genotyping in additional understudied areas is imperative.

The objectives of this study are to isolate *Bd* strains in Central Texas, characterize their genetic variation, and investigate genetic signatures of endemism and potentially recent introductions into the state. Texas is part of a region of the central and eastern United States in which there are no known chytridiomycosis-associated amphibian declines or extinctions. Although studies have confirmed the

presence of *Bd* in the eastern (Saenz et al. 2010) and central (Gaertner et al. 2009) parts of the state, no strains from these regions had been genotyped prior to our study (James et al. 2015). While a lack of known *Bd*-related population declines or extinctions in the area could be an indication that the pathogen has a long local history, other factors, such as climate or host resistance, may be responsible for attenuating disease outbreaks in the region. A study of the genetics of the fungus in Texas is an important first step in understanding disease dynamics between amphibians and *Bd* in the region. Additionally, by characterizing strains in Texas and placing them in the context of a global panel of strains, we can gain a more complete evolutionary picture of the *Bd* group, and perhaps a better understanding of the origins of its most virulent lineages. A study of this pathogenic fungus in this part of the state has important conservation implications, as well. Texas is home to 16 endemic amphibian species, many of which are federally listed as species of concern, and all of which are found in either the Edwards Plateau of Central Texas or the coastal prairies of East Central Texas (Tipton et al. 2012). Knowledge of the strains present in these regions can inform our understanding of the potential disease risks to these species in the face of continued climate change and habitat alteration.

II. METHODS

Field Collection and Sequencing

We collected anuran larvae from ponds in Travis, Hays, Bastrop, Kaufman, and Houston counties in central and eastern Texas (Table 1) and stored them in Falcon tubes or Tupperware containers with water from the collection site. We transported all collected tadpoles to the lab at Texas State University where they were euthanized. Each tadpole was given a unique identifier, and a tail clipping from each tadpole was preserved in 95% ethanol for later DNA extraction. We then excised the keratinized mouthparts (jaw sheaths and tooth rows) from each tadpole and transferred them to 1% agar petri dishes. We cleaned the mouthparts by dragging them through the agar and then transferred them to 1% tryptone agar petri dishes inoculated with penicillin-G and streptomycin sulfate for cultivation of *Bd*. We monitored the plates for growth of zoosporangia and active zoospores for up to two weeks. Once a sufficient number of active zoospores were observed, we removed a section of the agar containing active zoospores to tryptone broth (16 g tryptone in 1,000 ml deionized water, autoclaved). Each *Bd* isolate was labeled with the same identifier as the tadpole from which it was isolated. Once there was a high density of active zoospores in the broth, we transferred a 1-ml aliquot of broth from each isolate to a 1.5-ml centrifuge tube. We centrifuged the 1-ml aliquots of broth until a pellet of zoosporangia and zoospores could be seen at the bottom of each centrifuge tube. We discarded the supernatant and extracted DNA from the pellet using the Mammalian Tissue and Rodent Tail Genomic DNA Purification Protocol in the Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721,

#K0722 (Thermo Fisher Scientific, Inc.). We gauged extraction success with gel electrophoresis using a 1% agarose gel and 1x TBE buffer.

We performed conventional Polymerase Chain Reaction (PCR) on DNA from each successfully extracted isolate, using primers for previously described MLST loci (8009X2, BdC5, BdSc2.0, R6046, BdSC6.15) (Morehouse et al. 2003; Morgan et al. 2007; James et al. 2009; Schloegel et al. 2012; Jenkinson et al. 2016) (Table 3). Amplifications were performed in 25-µl volumes consisting of 12.5-µl DreamTaq PCR Master Mix (2x) (Thermo Fisher Scientific, Inc.), 11.5-µl nuclease free water, 0.25-µl forward primer, 0.25-µl reverse primer, and 0.5-µl DNA extraction. Thermocycling conditions consisted of a denaturing step of 2 min at 95°C, then 32 cycles of 30 s at 95°C, 30 s at 54°C (or 60°C, depending on primer), 45 s at 72°C, with a final extension of 10 min at 72°C. We treated 5-µl aliquots of PCR product with 2-µl ExoSAP-IT (USB Corp.) and incubated the total volume at 37°C for 30 min. Using each of the primers listed in Table 3, we performed cycle sequencing reactions using Big Dye v3.1 dye terminator (Applied Biosystems, Inc.). We incubated hydrated G-50 Sephadex (2.6 g/45 µl H_2O) at room temperature for 30 min, pipetted 400-µl aliquots into individual wells in a filter plate, and centrifuged the plate at 3000 rpm for 2 minutes to create a matrix through which cycle sequenced products were passed for purification. We dehydrated the purified cycle sequenced products, treated them with 12-µl formamide, and incubated them for 3 min at 104°C. We electrophoresed the cycle sequenced products on an ABI 3100-Avant genetic analyzer (Applied Biosystems, Inc.) and trimmed and edited the chromatograms using Geneious v9.1.5 (Kearse et al. 2012).

Cryopreservation

We used a cryoprotectorant solution consisting of 80 ml tryptone broth, 10 ml dimethyl sulfoxide, and 10 ml fetal calf serum to preserve all *Bd* isolates (Boyle et al. 2003). For each isolate, we centrifuged tryptone broth cultures in 1-ml tubes, discarded supernatant, and added 600 µl cryoprotectorant. All tubes were labeled with the appropriate identifier and stored in a freezer at -80°C.

Host Sequencing

We extracted mitochondrial DNA from tail muscle of all collected tadpoles using the same extraction protocol described for *Bd*. Conventional PCR was performed to amplify the cytochrome c oxidase I (COX1) gene, and PCR products were purified and sequenced using the same methods described for *Bd* sequencing. Chromatograms were trimmed and edited using Geneious v9.1.5 (Kearse et al. 2012). We identified the host species for each sample by using reference sequences from NCBI's GenBank database (Clark et al. 2016) and constructing a Bayesian phylogeny using MRBAYES v3.2.6 (Huelsenbeck & Ronquist 2001) on the CIPRES Science Gateway portal (Miller et al. 2010).

Data Analysis

We genotyped strains using reference sequences from Jenkinson et al. (2016). To identify genetic structure among *Bd* isolates, we used STRUCTURE (Pritchard et al. 2000) with values of *K* ranging from 1 to 12 and 5 iterations per *K*, using 500,000 Markov Chain Monte Carlo repetitions with a burn-in of 100,000. We determined the best number of populations by calculating ΔK (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl & von Holdt 2012) and obtained an average of our iterations per *K* using the program CLUMPP (Jakobsson & Rosenberg 2007). We performed an initial

analysis with only our Texas isolates, followed by a second analysis combining the Texas isolates with a set of globally distributed isolates that had been sequenced and genotyped using at least four of the same markers in previous studies (Schloegel et al. 2012; Jenkinson et al. 2016). Additionally, we performed a principal components analysis (PCA) using R packages ADE4 (Dray & Dufour 2007) and ADEGENET (Jombart 2008) to visualize genotype clusters of Texas isolates with the set of globally distributed isolates. We removed identical genotypes from the same region from our dataset before running both PCA and STRUCTURE analyses.

We used R packages ADEGENET (Jombart 2008), ADE4 (Dray & Dufour 2007), PEGAS (Paradis 2010), and HIERFSTAT (de Meeûs & Goudet 2007) to calculate measures of genetic diversity among our Texas isolates and compare them to other globally distributed isolates. We calculated these statistics for two datasets based on the number of available sequenced MLST loci. One dataset included 5 MLST loci and 3 putative populations—GPL populations from Texas and Brazil, as well as the endemic Brazilian lineage. The second dataset included a broader range of globally distributed isolates with 4 MLST loci. We calculated global and per population values of gene diversity, or expected heterozygosity (H_E), observed heterozygosity (H_O), and F_{IS} for each dataset. We also performed Hardy Weinberg tests to determine if the populations deviated from Hardy Weinberg expectations. We then calculated pairwise F_{ST} values to determine the level of differentiation between putative populations.

III. RESULTS

We attempted to isolate *Bd* from a total of 140 tadpoles collected from sites in Central, East, and North Central Texas (Table 2, Figure 1). From this total, we successfully cultured 42 chytrid isolates from 41 individual tadpoles comprising three host species (Table 2). A total of 37 isolates (88%) have been characterized using all 5 of our MLST loci. We were unable to amplify DNA from three isolates from two *R. clamitans* larvae using these markers, so we then amplified and sequenced DNA at the small subunit of the 18S ribosomal RNA (rRNA) gene. Using the NCBI BLAST search tool (Clark et al. 2016), we identified two of these isolates as *Rhizophlyctis harderi* (which has recently been re-named *Uebelmesseromyces harderi* (Powell et al. 2015)), and the other as *Chytriomyces hyalinus*, both of which are species of chytrids not known to infect vertebrate hosts. A few additional plates were discarded due to fungal or antibiotic-resistant bacterial contamination, so a small number of additional isolates may have been lost to this contamination. We have been unable to sequence two additional isolates and are currently working to identify these using the 18S rRNA gene. Of our 37 *Bd* isolates, there were 19 unique multi-locus genotypes (MLGs), and no MLGs were found at more than one collection site.

Host Identification

We used morphological characters to identify all tadpoles collected in our study as ranids, or true frogs. To identify tadpoles at the species level, we sequenced all specimens at a region of the COI gene and performed a Bayesian phylogenetic analysis using *Rana muscosa* (Accession # KU985709.1) as an outgroup and *Rana clamitans* (Accession # KY587195.1) and *Rana sphenocephala* (Accession # KT388406.1) as

reference sequences. All sequences were taken from the NCBI GenBank database (Clark et al. 2016). We chose these reference sequences because we suspected that some of our tadpoles belonged to these species based on geographic distribution. Although we also suspected that some of our specimens were *Rana berlandieri*, a COI reference sequence for this species was unavailable in GenBank. Our Bayesian phylogeny (Figure 2) revealed three distinct clades among our collected specimens, with two of these clades grouping with the *R. clamitans* and *R. sphenocephala* reference sequences, respectively. We identified the third clade as *Rana berlandieri* based on its genetic proximity to *R. sphenocephala*, the geographic location of the collection sites from which these specimens were taken, and the morphology of adult frogs observed at some of these sites.

Cluster Analyses

We performed a STRUCTURE analysis on the 19 unique MLGs from Texas and failed to detect population structure among these strains. We then conducted a STRUCTURE analysis and a PCA using a combined dataset that included our Texas isolates, as well as isolates that had been genotyped in two previous studies (Schloegel et al. 2012; Jenkinson et al. 2016). For these analyses, we were limited to using the 4 MLST loci (r6046, BdSc6.15, 8009x2, and BdC5) that had been sequenced in all three studies. Because of this, the number of unique MLGs from Texas used in these analyses was reduced to 13.

Our STRUCTURE analysis indicated 2-3 genetically distinct populations in our dataset, with the highest ΔK occurring at K=2, and a significantly high ΔK at K=3 (Figures 3A-B). By using the already genetically identified isolates from the two previous studies as references, we determined that, at K=2, our clusters corresponded to

Bd-GPL and *Bd*-Brazil, while at *K*=3, substructure within the GPL was revealed that corresponded to the two major clades within this group. No Texas isolates were assigned to the cluster representing *Bd*-Brazil. Nine of the 13 isolates showed at least a 79% probability of belonging to *Bd*-GPL1. Two isolates were ambiguous, with a 20.3% or less difference in support for the GPL1 and GPL2. The final two isolates had a 71.7% and 88.2% probability of belonging to *Bd*-GPL2.

The first three principal components of our PCA using Texas and global genotypes accounted for 81.5% of the total variance in the data, explaining 36.1%, 28.1%, and 17.4% of the variance, respectively (Figure 4). PC1 illustrates the differentiation between the GPL and *Bd*-Brazil, with two hybrid strains falling near the midpoint of the PC1 axis. Differentiation between the GPL1 and GPL2 is largely explained by PC2. The PCA shows minimal differentiation between isolates from Texas and other regions of North America, with most of these isolates clustering with the GPL1.

Measures of Genetic Variation

We calculated measures of genetic diversity for two datasets—one dataset including all unique genotypes from our study, from Jenkinson et al. (2016), and from Schloegel et al. (2012) using the 4 MLST loci common to all three of these studies, and one dataset including all unique genotypes from our study and from Jenkinson et al. (2016) using the 5 MLST loci common to the two studies (Table 4). The hybrid strains from Jenkinson et al. (2016) and Schloegel et al. (2012) were excluded from these analyses. The 4 MLST loci dataset included isolates from Texas, the rest of North America, Brazil, Panama, Africa, and Australia, while the 5 MLST loci dataset included isolates from Texas and Brazil.

In the 4 MLST loci dataset, we report data from only four populations (Texas GPL isolates, other North American GPL isolates, Brazil GPL isolates, and *Bd*-Brazil), because sample sizes from Panama, Africa, and Australia were low (Table 5). However, isolates from these four populations reflect a broad range of *Bd* diversity, encompassing both GPL lineages as well as the endemic Brazilian lineage. While global gene diversity, or expected heterozygosity (0.464), was higher than global observed heterozygosity (0.375), all but one population (the North American GPL) had higher observed heterozygosity than gene diversity when analyzed separately. Gene diversity was noticeably high in isolates from Texas (0.379) and the rest of North America (0.422) compared to GPL isolates from Brazil (0.276) and *Bd*-Brazil (0.261), while observed heterozygosity was less differentiated across populations, with Texas isolates ranking highest (0.421) and GPL isolates from Brazil lowest (0.381). A striking finding in our data was the much smaller difference between gene diversity and observed heterozygosity in isolates from Texas and the rest of North America compared to isolates from both Brazilian populations.

While the global F_{IS} value for the 4 MLST loci dataset was positive, all populations except for North America had negative F_{IS} values, indicating greater than expected heterozygosity under Hardy Weinberg Equilibrium in these populations. Populations from Brazil had highly negative F_{IS} values, reflecting the much greater difference between observed heterozygosity and gene diversity in these populations. However, Hardy Weinberg tests revealed insignificant *p* values for all populations, indicating that these deviations from expectation are not statistically significant.

In the 5 MLST loci dataset (results in Table 6), which consisted of three populations (Texas GPL isolates, Brazil GPL isolates, and *Bd*-Brazil isolates), global gene diversity was 0.506, and observed heterozygosity was 0.410. However, when these measures were calculated separately for each population, H_O was greater than H_E in all populations. Gene diversity was highest among Texas isolates (0.393), while observed heterozygosity was highest among GPL isolates from Brazil (0.428). *Bd*-Brazil had both the lowest gene diversity (0.296) and observed heterozygosity (0.343) among the three groups of isolates. As seen in the 4 MLST loci dataset, there was less differentiation between gene diversity and observed heterozygosity in Texas isolates compared to those from Brazil. The global F_{IS} for this dataset was positive (0.190), but each population had a negative F_{IS} value when analyzed independently. As with the 4 MLST loci dataset, results of Hardy Weinberg tests were insignificant for all populations.

Pairwise F_{ST} values were calculated for both datasets, but because we did not see a striking difference in values between datasets, we report only the results from the 4 MLST loci dataset here (Figure 5). F_{ST} values indicate much greater differentiation between *Bd*-Brazil and all other populations, while Texas isolates show no appreciable difference from other North American isolates (pairwise $F_{ST} = 0.032$) (Figure 5).

IV. DISCUSSION

A primary objective of this study was to investigate genetic signatures of diversity that would support a determination between introduction or endemism in *Bd* strains from Central Texas. Evidence supporting endemism includes signs of genetic structure across geography, indicating the evolution of distinct populations in different regions. A lack of genetic structure across geography would be expected with a recently introduced organism. Our analyses lend support to the novel pathogen hypothesis in North America. We were unable to detect population structure among the *Bd* isolates we collected, and were also unable to detect significant genetic differentiation between *Bd* isolates from Texas and those from elsewhere in North America. All isolates collected in this study belong to the widely distributed GPL, and the majority of these isolates appear to belong to the GPL1. One Texas isolate likely belongs to the GPL2, and three other isolates have some degree of statistical evidence supporting their placement in this clade as well. Evidence for hybridization between *Bd* lineages has been established (Schloegel et al. 2012; Jenkinson et al. 2016), thus it is possible that these ambiguous strains could be the result of hybridization between the two GPL lineages. Alternatively, these ambiguities could merely reflect a lack of resolution in our data that could be addressed with the use of additional markers. The presence of the GPL2 in Texas is not a surprising discovery given that GPL2 isolates have also been documented in South Carolina, Ohio, and Colorado (Schloegel et al. 2012), but its apparent rarity relative to the GPL1 is consistent with the pattern seen across the North American continent (James et al. 2015).

Similarity can also be observed between Texas and other North American *Bd* isolates in measures of gene diversity (Texas, 0.379; North America, 0.422) and observed

heterozygosity (Texas, 0.421; North America, 0.382). Although F_{IS} values differ in that Texas isolates have a slightly negative value (-0.085) and other North American isolates have a slightly positive value (0.096), results of Hardy Weinberg tests for both populations were insignificant. This result might be considered surprising for an organism that primarily reproduces clonally. Clonal reproduction should lead to an excess of heterozygosity and highly negative F_{IS} values (Jenkinson et al. 2016). However, loss of heterozygosity can be achieved through mitotic recombination and sexual recombination, both of which are thought to occur at least occasionally in *Bd* (James et al. 2009; Rosenblum et al. 2013; Jenkinson et al. 2016). It is possible that these recombination events have contributed to the patterns seen in our data, but the question remains as to why this lack of heterozygote excess occurs in North America and not in Brazil. Another interesting finding in our data is the relatively high gene diversity in *Bd* isolates from Texas and the rest of North America compared to isolates from Brazil. Currently, we can only speculate as to why this might be the case. Perhaps the GPL1 is a more genetically diverse clade compared to the GPL2 and *Bd*-Brazil, although it would seem counterintuitive for any clade of the GPL to be more genetically diverse than the older, basal *Bd*-Brazil lineage. Another possibility is that different selection pressures in temperate versus tropical climates are driving the observed differences in gene diversity in these two distinct regions of the world. However, we advise caution in drawing conclusions from these data until genome wide estimates are performed. While F_{IS} values for Brazilian isolates were highly negative, Hardy Weinberg tests for both *Bd*-Brazil and the GPL in Brazil also yielded insignificant results, a finding that conflicts with results from Jenkinson et al. (2016). These authors incorporated 12 MLST loci in their analyses,

compared to the 5 loci used in our study, and obtained highly significant *p* values from their Hardy Weinberg tests. The difference in the number of markers used may explain the differences in our results. We recommend conducting future analyses with whole genome data to eliminate potential bias in our data resulting from the use of a few selected markers.

Results of our study are consistent with the novel pathogen hypothesis. *Bd* strains in Texas are genetically similar to strains found throughout North America, including strains responsible for severe outbreaks of chytridiomycosis in the western United States. This finding leaves us with a still unanswered question—why do these strains, which are genetically similar, cause disease and mortalities in some regions but not others? If the pathogen is the same in these different regions, the other two points of the epidemiological triangle—host and environment—must be explored further. One intuitive explanation for the lack of known chytridiomycosis outbreaks in Texas is the influence of climate. The most devastating *Bd*-associated population declines have occurred in cool, high-elevation regions (Berger et al. 1998; Lips et al. 2006; Bosch et al. 2001; Vredenburg et al. 2010), and Piotrowski et al. (2004) reported *Bd*'s optimal temperature range to be 17-25°C with a maximum threshold of 28°C. In Texas, amphibians may be able to clear *Bd* infections before they become lethal coincident to hot summers, when temperatures regularly exceed 32°C (Nielsen-Gammon 2011). We conjecture that *Bd* is able to persist during these warm seasons in refugia and then re-infect hosts as the weather becomes cooler. The cyclical nature of these weather conditions may serve to keep severe disease outbreaks in check, in contrast to regions that are thermally stable and cool year-round.

However, weather conditions cannot explain the lack of *Bd*-associated morbidity in Central Texas salamanders of the genus *Eurycea*, which dwell in cool, thermally stable aquifers and springs. While *Bd* infections in these salamanders have been documented by Gaertner et al. (2009), symptoms of chytridiomycosis have not been detected in these populations despite many years of investigation (Bowles et al. 2006; Pierce et al. 2014; Bendik 2017). Differences in host resistance among and even within species have been documented (Woodhams et al. 2007; Savage & Zamudio 2011), so it is possible that host species immunity plays a role in limiting the severity of disease outcomes in Central Texas. Further study will be necessary to fully understand the effects of both climate and host resistance on the dynamics of *Bd* infection in this region.

Non-Batrachochytrium Chytrids Infecting Amphibians?

A striking finding from our isolation and culturing efforts was the identification of two chytrid species—*Uebelmesseromyces harderi* and *Chytriomyces hyalinus*—not known to infect vertebrate hosts. Chytrids, or zoosporic fungi belonging to a group known as the Chytridiomycota, are generally saprobes or parasites of algae, plants, and invertebrates (Barr, 2001). *Bd* and its only known congener, *Batrachochytrium salamandrivorans* (or *Bsal*), a more host specific *Batrachochytrium* that targets salamanders, are the only chytrids known to infect vertebrates (Martel et al. 2013). If, in fact, *Uebelmesseromyces harderi* and *Chytriomyces hyalinus* are discovered to infect amphibians, this would be a significant finding. However, we must first satisfy Koch's postulates and demonstrate that these species can infect healthy, previously unexposed amphibians before we can make this claim (Fredricks & Relman 1996). It is possible that these chytrids were present in the pond from which these tadpoles were collected, and

that they somehow persisted on the tadpole mouthparts despite our efforts to clean them by dragging them through agar. It does not seem unreasonable, however, to expect that more chytrids may infect amphibian and other vertebrate species than we currently realize. Although studies of the morphology and systematics of *U. harderi* and *C. hyalinus* can be found in the literature, little appears to be known about the life history of these species (Letcher & Powell 2002; Powell et al. 2015). The fact that *Bd* was only first discovered and described in the 1990s and *Bsal* was described in 2013 highlights that parasitic chytrids have easily escaped our attention until they noticeably impact other species. Thus, the possibility that *U. harderi* and *C. hyalinus* might infect amphibians is intriguing and demanding of further study.

Future Directions

The strains identified and preserved in this study will lay the foundation for future work. Although thermal tolerance ranges for *Bd* have been established (Piotrowski et al. 2004), more recent work has identified variation in thermal tolerance among strains (Stevenson et al. 2013). Texas strains will provide ideal study subjects for testing the possibility that *Bd* can evolve higher thermal tolerances in exceptionally warm climates. Environmental factors and host tolerance can be further explored by subjecting strains and hosts to differential environmental conditions and comparing resulting infection prevalence and severity, and by infection studies with different host species. We also intend to conduct whole genome analyses of these isolates. Genomic analyses would allow us to more confidently place our isolates in the broader evolutionary context of this globally distributed group, and may also serve to resolve some of the ambiguities in our current data.

Table 1. All Tadpoles Collected for this Study with Collection Number, Species, Isolate Number and Species, County of Collection, and Site Name in Texas, USA.

Tadpole	Tadpole Species	Isolate ID	Species/Strain	County	Site
TM001	*R. sphenocephala*			Bastrop	GLR
TM002	*R. sphenocephala*			Bastrop	GLR
TM003	*R. sphenocephala*			Bastrop	GLR
TM004	*R. sphenocephala*			Bastrop	GLR
TM005	*R. sphenocephala*			Bastrop	GLR
TM006	*R. sphenocephala*			Bastrop	GLR
TM007	*R. berlandieri*			Hays	Abby's Pond
TM008	*R. berlandieri*			Hays	Abby's Pond
TM009	*R. berlandieri*			Hays	Abby's Pond
TM010	*R. clamitans*			Houston	DCNF
TM011	*R. clamitans*			Houston	DCNF
TM012	*R. clamitans*			Houston	DCNF
TM013	*R. clamitans*			Houston	DCNF
TM014	*R. clamitans*			Houston	DCNF
TM015	*R. berlandieri*	TXST001	*Bd*-GPL1	Hays	Abby's Pond
TM016	*R. berlandieri*	TXST002	*Bd*-GPL1	Hays	Abby's Pond
TM017	*R. berlandieri*	TXST003	*Bd*-GPL1	Hays	Abby's Pond
TM018	*R. sphenocephala*			Kaufman	RRR Ranch
TM019	*R. sphenocephala*			Kaufman	RRR Ranch
TM020	*R. sphenocephala*	TXST004	*Bd*-GPL	Kaufman	RRR Ranch
TM021	*R. sphenocephala*			Kaufman	RRR Ranch
TM022	*R. sphenocephala*			Kaufman	RRR Ranch
TM023	*R. sphenocephala*	TXST005	*Bd*-GPL1	Kaufman	RRR Ranch
TM024	*R. sphenocephala*			Kaufman	RRR Ranch
TM025	*R. sphenocephala*			Kaufman	RRR Ranch

Table 1. All Tadpoles Collected for this Study with Collection Number, Species, Isolate Number and Species, County of Collection, and Site Name in Texas, USA. *Continued.*

TM026	*R. sphenocephala*	TXST006	*Bd*-GPL1	Kaufman	RRR Ranch
TM027	*R. sphenocephala*			Kaufman	RRR Ranch
TM028	*R. sphenocephala*	TXST007	*Bd*-GPL1	Kaufman	RRR Ranch
TM029	*R. sphenocephala*	TXST008	*Bd*-GPL1	Kaufman	RRR Ranch
TM030	*R. sphenocephala*	TXST009	*Bd*-GPL1	Kaufman	RRR Ranch
TM031	*R. sphenocephala*			Kaufman	RRR Ranch
TM032	*R. sphenocephala*	TXST010	*Bd*-GPL1	Kaufman	RRR Ranch
TM033	*R. sphenocephala*	TXST011	*Bd*-GPL	Kaufman	RRR Ranch
TM034	*R. sphenocephala*	TXST012	*Bd-GPL2*	Kaufman	RRR Ranch
TM035	*R. sphenocephala*			Kaufman	RRR Ranch
TM036	*R. sphenocephala*	TXST013	*Bd*-GPL1	Kaufman	RRR Ranch
TM037	*R. berlandieri*			Travis	Bee Cave
TM038	*R. berlandieri*	TXST014	*Bd*-GPL1	Travis	Bee Cave
TM039	*R. berlandieri*	TXST015	*Bd*-GPL1	Travis	Bee Cave
TM040	*R. berlandieri*			Travis	Bee Cave
TM041	*R. berlandieri*	TXST016	*Bd*-GPL1	Hays	Wimberley
TM042	*R. berlandieri*			Hays	Wimberley
TM043	*R. berlandieri*			Hays	Wimberley
TM044	*R. berlandieri*			Hays	Wimberley
TM045	*R. sphenocephala*	TXST017	*Bd*-GPL1	Kaufman	RRR Ranch
TM046	*R. sphenocephala*			Kaufman	RRR Ranch
TM047	*R. sphenocephala*			Kaufman	RRR Ranch
TM048	*R. sphenocephala*	TXST018	*Bd*-GPL1	Kaufman	RRR Ranch
TM049	*R. sphenocephala*	TXST019	*Bd*-GPL1	Kaufman	RRR Ranch
TM050	*R. sphenocephala*			Kaufman	RRR Ranch
TM053	*R. sphenocephala*			Kaufman	RRR Ranch
TM054	*R. sphenocephala*			Kaufman	RRR Ranch

Table 1. All Tadpoles Collected for this Study with Collection Number, Species, Isolate Number and Species, County of Collection, and Site Name in Texas, USA. *Continued.*

TM055	*R. sphenocephala*			Kaufman	RRR Ranch
TM056	*R. sphenocephala*			Kaufman	RRR Ranch
TM057	*R. sphenocephala*			Kaufman	RRR Ranch
TM058	*R. sphenocephala*			Kaufman	RRR Ranch
TM059	*R. sphenocephala*			Kaufman	RRR Ranch
TM060	*R. sphenocephala*			Kaufman	RRR Ranch
TM061	*R. sphenocephala*			Kaufman	RRR Ranch
TM062	*R. sphenocephala*			Kaufman	RRR Ranch
TM063	*R. sphenocephala*	TXST038	Unknown	Kaufman	RRR Ranch
TM064	*R. sphenocephala*			Kaufman	RRR Ranch
TM065	*R. sphenocephala*			Kaufman	RRR Ranch
TM066	*R. sphenocephala*			Kaufman	RRR Ranch
TM067	*R. sphenocephala*			Kaufman	RRR Ranch
TM068	*R. sphenocephala*			Kaufman	RRR Ranch
TM069	*R. sphenocephala*			Kaufman	RRR Ranch
TM070	*R. sphenocephala*			Kaufman	RRR Ranch
TM071	*R. sphenocephala*			Kaufman	RRR Ranch
TM072	*R. sphenocephala*			Kaufman	RRR Ranch
TM074	*R. berlandieri*	TXST020	*Bd*-GPL1	Travis	BFL
TM075	*R. berlandieri*	TXST021	*Bd*-GPL1	Travis	BFL
TM076	*R. berlandieri*	TXST022	*Bd*-GPL1	Travis	BFL
TM077	*R. berlandieri*			Travis	BFL
TM078	*R. berlandieri*			Travis	BFL
TM079	*R. berlandieri*			Travis	BFL
TM080	*R. berlandieri*			Travis	BFL
TM081	*R. berlandieri*	TXST023	*Bd*-GPL1	Travis	BFL
TM082	*R. berlandieri*			Travis	BFL

Table 1. All Tadpoles Collected for this Study with Collection Number, Species, Isolate Number and Species, County of Collection, and Site Name in Texas, USA. *Continued.*

TM083	*R. berlandieri*			Travis	BFL
TM084	*R. berlandieri*			Travis	BFL
TM085	*R. berlandieri*			Travis	BFL
TM086	*R. berlandieri*	TXST024	*Bd*-GPL1	Travis	BFL
TM087	*R. berlandieri*			Travis	BFL
TM088	*R. berlandieri*	TXST025	*Bd*-GPL1	Travis	BFL
TM089	*R. berlandieri*	TXST039	Unknown	Travis	BFL
TM090	*R. berlandieri*	TXST026	*Bd*-GPL	Travis	BFL
TM091	*R. berlandieri*	TXST027	*Bd*-GPL1	Travis	Lakeway
TM092	*R. berlandieri*	TXST028	*Bd*-GPL1	Travis	Lakeway
TM093	*R. berlandieri*	TXST029	*Bd*-GPL1	Travis	Lakeway
TM094	*R. berlandieri*	TXST030	*Bd*-GPL1	Travis	Lakeway
TM095	*R. berlandieri*	TXST031	*Bd*-GPL1	Travis	Lakeway
TM096	*R. berlandieri*	TXST032	*Bd*-GPL1	Travis	Lakeway
TM097	*R. berlandieri*			Travis	BFL
TM098	*R. clamitans*			Houston	DCNF
TM099	*R. clamitans*			Houston	DCNF
TM100	*R. clamitans*			Houston	DCNF
TM101	*R. clamitans*			Houston	DCNF
TM102	*R. clamitans*			Houston	DCNF
TM103	*R. clamitans*			Houston	DCNF
TM104	*R. clamitans*			Houston	DCNF
TM105	*R. clamitans*			Houston	DCNF
TM106	*R. clamitans*			Houston	DCNF
TM107	*R. clamitans*			Houston	DCNF
TM108	*R. clamitans*	TXST040	*Uebelmesseromyces harderi*	Houston	DCNF
TM109a	*R. clamitans*	TXST041	*Chytriomyces hyalinus*	Houston	DCNF

Table 1. All Tadpoles Collected for this Study with Collection Number, Species, Isolate Number and Species, County of Collection, and Site Name in Texas, USA. *Continued.*

TM109b	*R. clamitans*	TXST042	*Uebelmesseromyces harderi*	Houston	DCNF
TM110	*R. clamitans*			Houston	DCNF
TM111	*R. clamitans*			Houston	DCNF
TM112	*R. clamitans*			Houston	DCNF
TM113	*R. clamitans*			Houston	DCNF
TM114	*R. berlandieri*			Travis	BFL
TM115	*R. berlandieri*			Travis	BFL
TM116	*R. berlandieri*			Travis	BFL
TM117	*R. berlandieri*			Travis	BFL
TM118	*R. berlandieri*			Travis	BFL
TM119	*R. berlandieri*			Travis	BFL
TM120	*R. berlandieri*			Travis	BFL
TM121	*R. berlandieri*	TXST033	*Bd*-GPL1	Travis	BFL
TM122	*R. berlandieri*	TXST034	*Bd-GPL1*	Travis	BFL
TM123	*R. berlandieri*			Travis	BFL
TM124	*R. berlandieri*			Travis	BFL
TM125	*R. berlandieri*	TXST035	*Bd-GPL1*	Travis	BFL
TM126	*R. berlandieri*			Travis	BFL
TM127	*R. berlandieri*	TXST036	*Bd-GPL1*	Travis	Bee Cave
TM128	*R. berlandieri*	TXST037	*Bd-GPL1*	Travis	Bee Cave
TM134	*R. berlandieri*			Travis	BFL
TM135	*R. berlandieri*			Travis	BFL
TM136	*R. berlandieri*			Travis	BFL
TM137	*R. berlandieri*			Travis	BFL
TM138	*R. berlandieri*			Travis	BFL
TM139	*R. berlandieri*			Travis	BFL
TM140	*R. berlandieri*			Travis	BFL
TM141	*R. berlandieri*			Travis	BFL

Table 2. Collection Sites with County (TX, USA), GPS Coordinates, Number of Tadpoles Collected, Number of Chytrid Strains Isolated (including non-*Bd* chytrids), and Tadpole Species.

Site	County	Latitude (N)	Longitude (W)	Tadpoles Collected (*N*)	Chytrid Isolates (*N*)	Tadpole Species
Abby's Pond	Hays	29.9772	-97.8984	6	3	*R. berlandieri*
Bee Cave	Travis	30.2542	-97.9392	6	4	*R. berlandieri*
Brackenridge Field Laboratory	Travis	30.2837	-97.7801	40	11	*R. berlandieri*
Davy Crockett National Forest	Houston	31.4083	-95.1667	21	3	*R. clamitans*
Griffith League Ranch	Bastrop	30.2148	-97.2576	6	0	*R. sphenocephala*
Lakeway	Travis	30.3766	-98.0421	6	6	*R. berlandieri*
RRR Ranch	Kaufman	32.5332	-96.4880	47	14	*R. sphenocephala*
Wimberley	Hays	29.9955	-98.2163	8	1	*R. berlandieri*
Total				140	42	

Table 3. Multi-Locus Sequence Typing (MLST) markers used to identify lineages of Texas *Bd* isolates with genomic contig and position, number of alleles, forward and reverse primers, annealing temperature, and source publication.

Locus	Genomic Contig and Position	Alleles	Primers	Annealing Temp.	Source
8009x2	SC 1: 0.64 Mbp	4	F: 5'-TCGTGAAGAGCTTGGAAAGTCG-3'	54°	Morgan et al. 2007
			R: 5'-AGTTCTGTCGTCAATGCTGTAGGG-3'		
BdC5	SC 1: 1.45 Mbp	3	F: 5'-TAATAGCGCCGACCGAACTA-3'	54°	James et al. 2009
			R: 5'-ATGCCAAACCATGAGCAAAT-3'		
BdSC2.0	SC 2: 0.06 Mbp	4	F: 5'-TCAAGGTGCGTTTGCTAGTG-3'	60°	Jenkinson et al. 2016
			R: 5'-GCACTTACTGTTGGCAGCTTT-3'		
R6046	SC 5: 1.22 Mbp	2	F: 5'-CTATCTGCGCTCCCGTGTCAA-3'	54°	Morehouse et al. 2003
			R: 5'-AGGGCTGCAACAACTGGATTT-3'		
BdSC6.15	SC 6: 1.51 Mbp	4	F: 5'-GACGATAAAACGACAACAATCG-3'	54°	Schloegel et al. 2012
			R: 5'-CCCTTTTTAGGTTGGCTTGC-3'		

Table 4. *Bd* Isolates Analyzed for this Study with Lineage, Location Collected, and Original Publication. Two datasets were derived from this collection of isolates. One dataset consisted of all isolates, which were analyzed at 4 MLST loci (8009x2, BdC5, R6046, BdSC6.15). A second dataset excluded all isolates from Schloegel et al. (2012) (marked in this table with an asterisk); the remaining isolates were analyzed separately using all 5 MLST loci listed in Table 3.

Isolate	Lineage	Location	Source
TXST001	GPL1	Texas	Collected for this study
TXST002	GPL1	Texas	Collected for this study
TXST003	GPL1	Texas	Collected for this study
TXST004	GPL	Texas	Collected for this study
TXST005	GPL1	Texas	Collected for this study
TXST007	GPL1	Texas	Collected for this study
TXST009	GPL1	Texas	Collected for this study
TXST012	GPL2	Texas	Collected for this study
TXST014	GPL1	Texas	Collected for this study
TXST015	GPL1	Texas	Collected for this study
TXST016	GPL1	Texas	Collected for this study
TXST017	GPL1	Texas	Collected for this study
TXST020	GPL1	Texas	Collected for this study
TXST021	GPL	Texas	Collected for this study
TXST023	GPL1	Texas	Collected for this study
TXST027	GPL1	Texas	Collected for this study
TXST029	GPL1	Texas	Collected for this study
TXST033	GPL1	Texas	Collected for this study
TXST036	GPL1	Texas	Collected for this study
CW34	GPL2	South Africa	Schloegel et al. 2012*
CW36	GPL2	South Africa	Schloegel et al. 2012*
JEL253	GPL2	Australia	Schloegel et al. 2012*
LB01	GPL2	Australia	Schloegel et al. 2012*
CLFT114	GPL2	Brazil	Jenkinson et al. 2016
CLFT048	GPL2	Brazil	Jenkinson et al. 2016
CLFT080	GPL2	Brazil	Jenkinson et al. 2016
CLFT082	GPL2	Brazil	Jenkinson et al. 2016
CLFT062	GPL2	Brazil	Jenkinson et al. 2016
CLFT032	GPL2	Brazil	Jenkinson et al. 2016
CLFT060	GPL2	Brazil	Jenkinson et al. 2016
CLFT130	GPL2	Brazil	Jenkinson et al. 2016
CLFT055	GPL2	Brazil	Jenkinson et al. 2016
CLFT021	GPL2	Brazil	Jenkinson et al. 2016
CLFT029	GPL2	Brazil	Jenkinson et al. 2016
CLFT030	GPL2	Brazil	Jenkinson et al. 2016
CLFT073	GPL2	Brazil	Jenkinson et al. 2016

Table 4. *Bd* Isolates Analyzed for this Study with Lineage, Location Collected, and Original Publication. *Continued*.

CLFT074	GPL2	Brazil	Jenkinson et al. 2016
CLFT081	GPL2	Brazil	Jenkinson et al. 2016
CLFT129	GPL2	Brazil	Jenkinson et al. 2016
CLFT033	GPL2	Brazil	Jenkinson et al. 2016
CLFT034	GPL2	Brazil	Jenkinson et al. 2016
CLFT133	GPL2	Brazil	Jenkinson et al. 2016
CLFT152	GPL2	Brazil	Jenkinson et al. 2016
LMS902	GPL2	Brazil	Jenkinson et al. 2016
LMS925	GPL2	Brazil	Jenkinson et al. 2016
CLFT054	GPL2	Brazil	Jenkinson et al. 2016
CLFT052	GPL2	Brazil	Jenkinson et al. 2016
CLFT099	GPL2	Brazil	Jenkinson et al. 2016
CLFT104	GPL2	Brazil	Jenkinson et al. 2016
CLFT132	GPL2	Brazil	Jenkinson et al. 2016
CLFT137	GPL2	Brazil	Jenkinson et al. 2016
CLFT116	GPL2	Brazil	Jenkinson et al. 2016
CLFT083	GPL2	Brazil	Jenkinson et al. 2016
CLFT105	GPL2	Brazil	Jenkinson et al. 2016
CLFT078	GPL2	Brazil	Jenkinson et al. 2016
CLFT101	GPL2	Brazil	Jenkinson et al. 2016
CLFT103	GPL2	Brazil	Jenkinson et al. 2016
CLFT095	GPL2	Brazil	Jenkinson et al. 2016
CLFT097	GPL2	Brazil	Jenkinson et al. 2016
CLFT131	GPL2	Brazil	Jenkinson et al. 2016
CLFT126	GPL2	Brazil	Jenkinson et al. 2016
CLFT087	GPL2	Brazil	Jenkinson et al. 2016
CLFT118	GPL2	Brazil	Jenkinson et al. 2016
CLFT079	GPL2	Brazil	Jenkinson et al. 2016
CLFT045	GPL2	Brazil	Jenkinson et al. 2016
CLFT085	GPL2	Brazil	Jenkinson et al. 2016
CLFT076	GPL2	Brazil	Jenkinson et al. 2016
CLFT086	GPL2	Brazil	Jenkinson et al. 2016
CLFT031	GPL2	Brazil	Jenkinson et al. 2016
CLFT047	GPL2	Brazil	Jenkinson et al. 2016
CLFT064	GPL2	Brazil	Jenkinson et al. 2016
CLFT051	GPL2	Brazil	Jenkinson et al. 2016
CLFT100	GPL2	Brazil	Jenkinson et al. 2016
CLFT053	GPL2	Brazil	Jenkinson et al. 2016
CLFT049	GPL2	Brazil	Jenkinson et al. 2016
CLFT058	GPL2	Brazil	Jenkinson et al. 2016
CLFT111	GPL2	Brazil	Jenkinson et al. 2016
CLFT077	GPL2	Brazil	Jenkinson et al. 2016

Table 4. *Bd* Isolates Analyzed for this Study with Lineage, Location Collected, and Original Publication. *Continued*.

CLFT138	GPL2	Brazil	Jenkinson et al. 2016
CLFT046	GPL2	Brazil	Jenkinson et al. 2016
CLFT043	GPL2	Brazil	Jenkinson et al. 2016
CLFT115	GPL2	Brazil	Jenkinson et al. 2016
CLFT035	GPL2	Brazil	Jenkinson et al. 2016
CLFT037	GPL2	Brazil	Jenkinson et al. 2016
CLFT123	GPL2	Brazil	Jenkinson et al. 2016
CLFT050	GPL2	Brazil	Jenkinson et al. 2016
CLFT036	GPL1	Brazil	Jenkinson et al. 2016
CLFT026	GPL2	Brazil	Jenkinson et al. 2016
CLFT136	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT141	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT143	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT148	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT040	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT044	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT061	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT065	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
JEL649	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT067	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT070	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT068	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
CLFT071	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
UM142	*Bd*-Brazil	Brazil	Jenkinson et al. 2016
JEL258	GPL1	North America	Schloegel et al. 2012*
JEL270	GPL1	North America	Schloegel et al. 2012*
JEL277	GPL1	North America	Schloegel et al. 2012*
JEL647	GPL1	North America	Schloegel et al. 2012*
JEL656	GPL1	North America	Schloegel et al. 2012*
JSOH-1	GPL2	North America	Schloegel et al. 2012*
SRS810	GPL2	North America	Schloegel et al. 2012*
JEL646	GPL1	North America	Schloegel et al. 2012*
JEL275	GPL2	North America	Schloegel et al. 2012*
JEL626	GPL1	North America	Schloegel et al. 2012*
JEL627	GPL1	North America	Schloegel et al. 2012*
JEL630	GPL1	North America	Schloegel et al. 2012*
PTH-001	GPL1	North America	Schloegel et al. 2012*
JEL230	GPL1	North America	Schloegel et al. 2012*
JEL644	GPL1	North America	Schloegel et al. 2012*
JEL213	GPL1	North America	Schloegel et al. 2012*
JEL289	GPL1	North America	Schloegel et al. 2012*
JEL360	GPL1	North America	Schloegel et al. 2012*

Table 4. *Bd* Isolates Analyzed for this Study with Lineage, Location Collected, and Original Publication. *Continued*.

JEL231	GPL1	North America	Schloegel et al. 2012*
PM5	GPL2	Panama	Schloegel et al. 2012*
PM1	GPL2	Panama	Schloegel et al. 2012*
JEL423	GPL2	Panama	Schloegel et al. 2012*

Table 5. Observed (H_O) and Expected Heterozygosity (H_E), F_{IS}, and Hardy Weinberg test *p* values with a 4 MLST dataset evaluating *Bd* strains genotyped across multiple studies, including those collected here, and strains from Schloegel et al. (2012) and Jenkinson et al. (2016).

Population	H_O	Gene Diversity (H_E)	F_{IS}	HW Test
Bd-GPL (Texas)	0.421	0.379	-0.112	0.548
Bd-GPL (North America)	0.382	0.422	0.096	0.673
Bd-GPL (Brazil)	0.381	0.276	-0.382	0.233
Bd-Brazil	0.339	0.261	-0.301	0.694
Global	0.375	0.464	0.192	<0.001

Table 6. Observed (H_O) and Expected Heterozygosity (H_E), F_{IS}, and Hardy Weinberg test *p* values with a 5 MLST dataset evaluating genotyped *Bd* strains collected for this study and those from Jenkinson et al. (2016).

Population	H_O	Gene Diversity (H_E)	F_{IS}	HW Test
Bd-GPL (Texas)	0.411	0.393	-0.044	0.524
Bd-GPL (Brazil)	0.428	0.319	-0.340	0.196
Bd-Brazil	0.343	0.296	-0.159	0.655
Global	0.412	0.504	0.183	<0.001

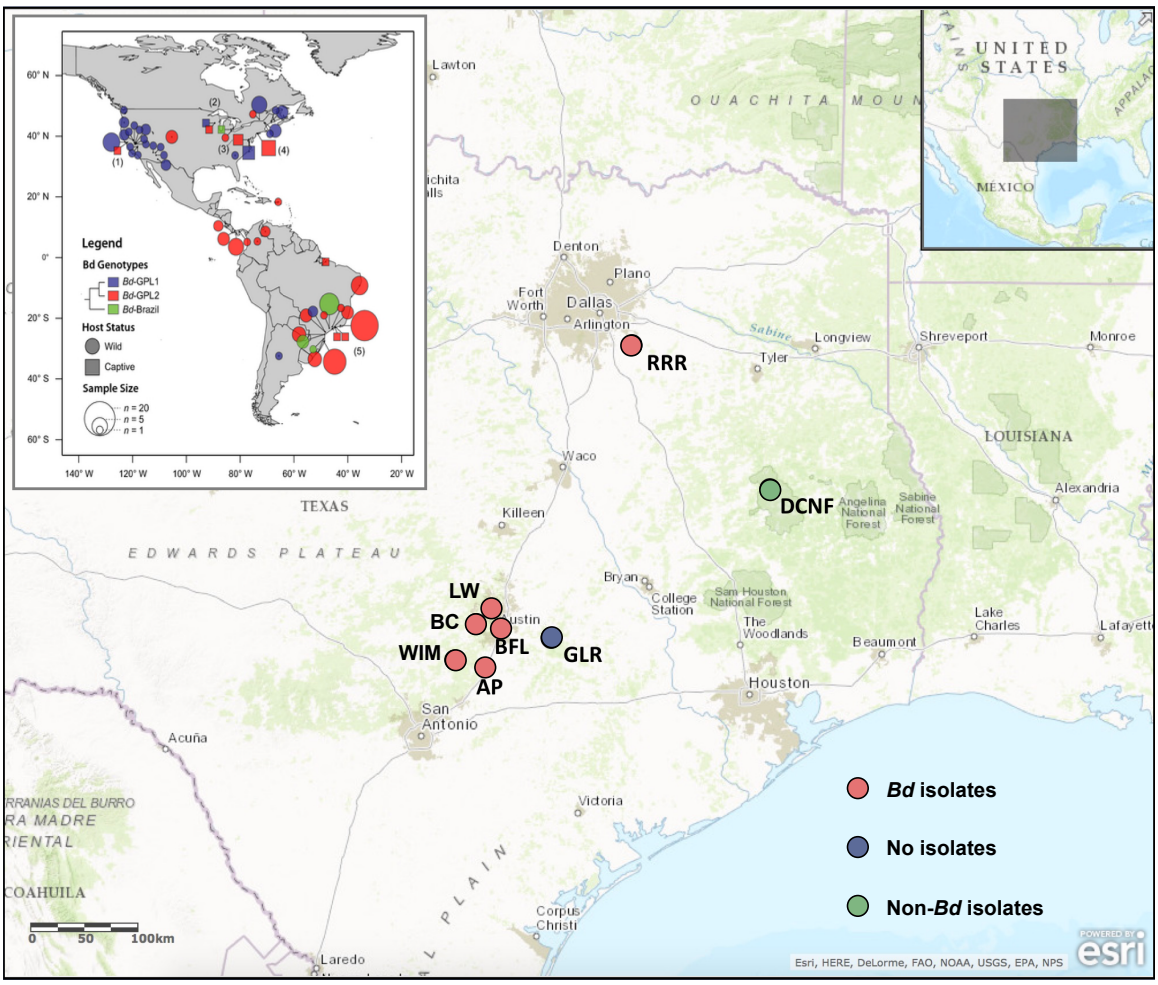

Figure 1. Collection Site Map and Context Within the Western Hemisphere. A map featuring all tadpole collection sites, labeled with site abbreviations and color-coded based on the type of isolates successfully cultured from each site. The inset map from Jenkinson et al. (2015) shows the localities and lineages of all genotyped *Bd* isolates from the Americas prior to this study.

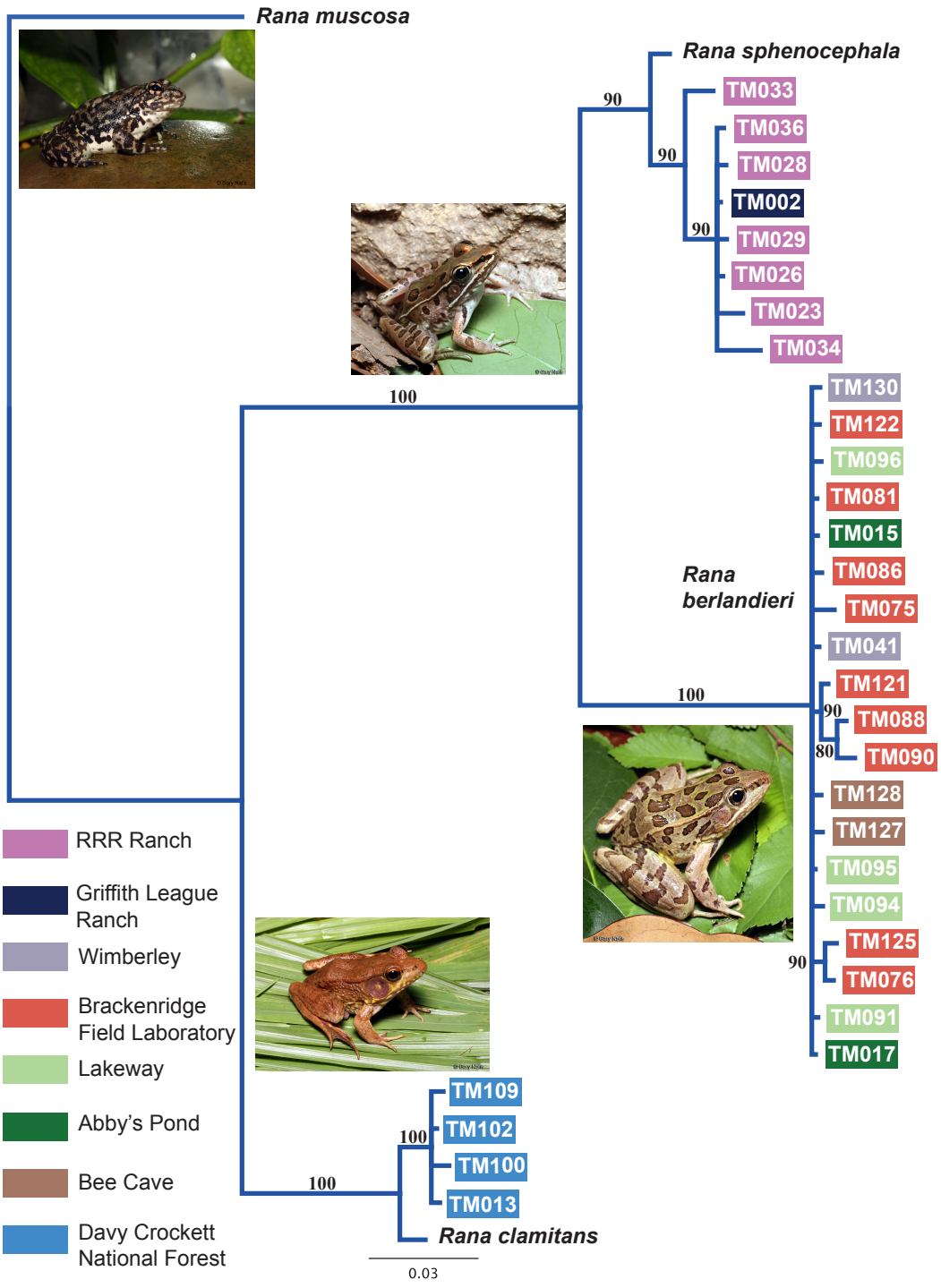

Figure 2. Phylogeny of Anuran Larvae Specimens. A Bayesian phylogeny of a subset of anuran larvae collected for this study, using the COI mitochondrial gene and produced with MRBAYES (default parameters) on the CIPRES Science Gateway portal. *Rana clamitans* (KY587195.1) and *Rana sphenocephala* (KT388406.1) sequences from GenBank were used as references to identify collected tadpoles, while *Rana berlandieri* specimens were identified by adult morphology, geography, and genetic proximity to *R. sphenocephala*. A *Rana muscosa* (KU985709.1) sequence was used as the outgroup. Photographs are by Gary Nafis and were downloaded from CaliforniaHerps.com.

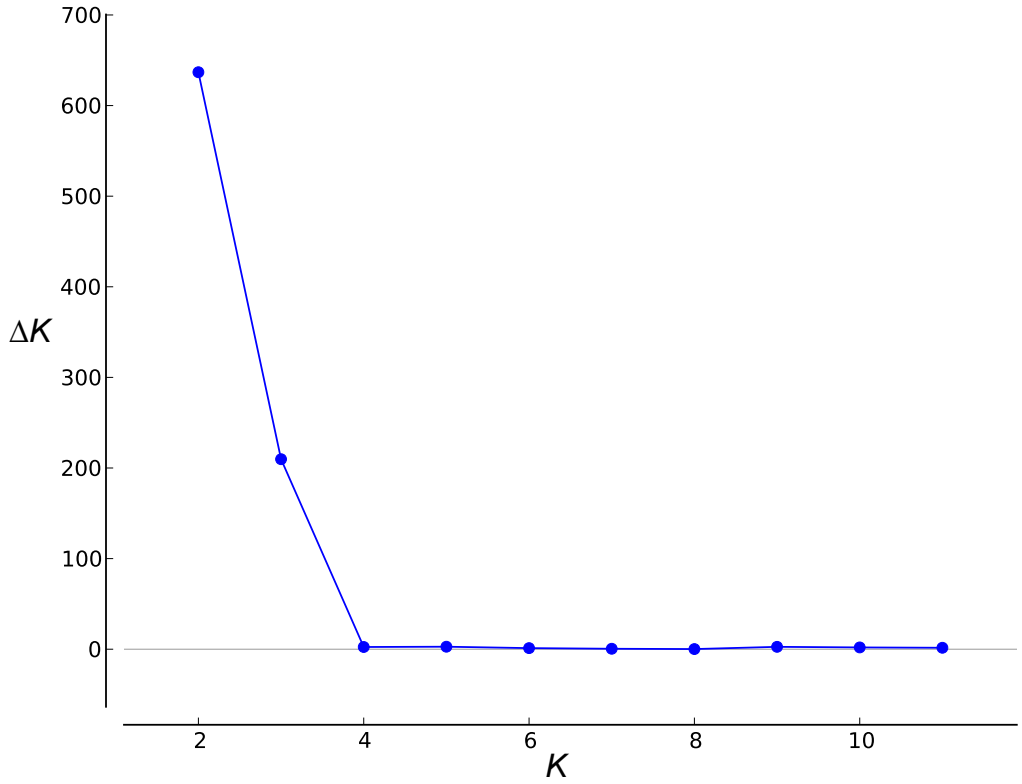

Figure 3A. The Evanno Method for Determining the Best *K*. Δ*K* for one to twelve clusters (*K*) based on five independent runs of STRUCTURE using a dataset of 45 *Bd* genotypes at 4 MLST loci, including 19 isolates collected across Texas (from anuran larvae of one genus and two species) for this study and globally distributed strains from Schloegel et al. (2012) and Jenkinson et al. (2016).

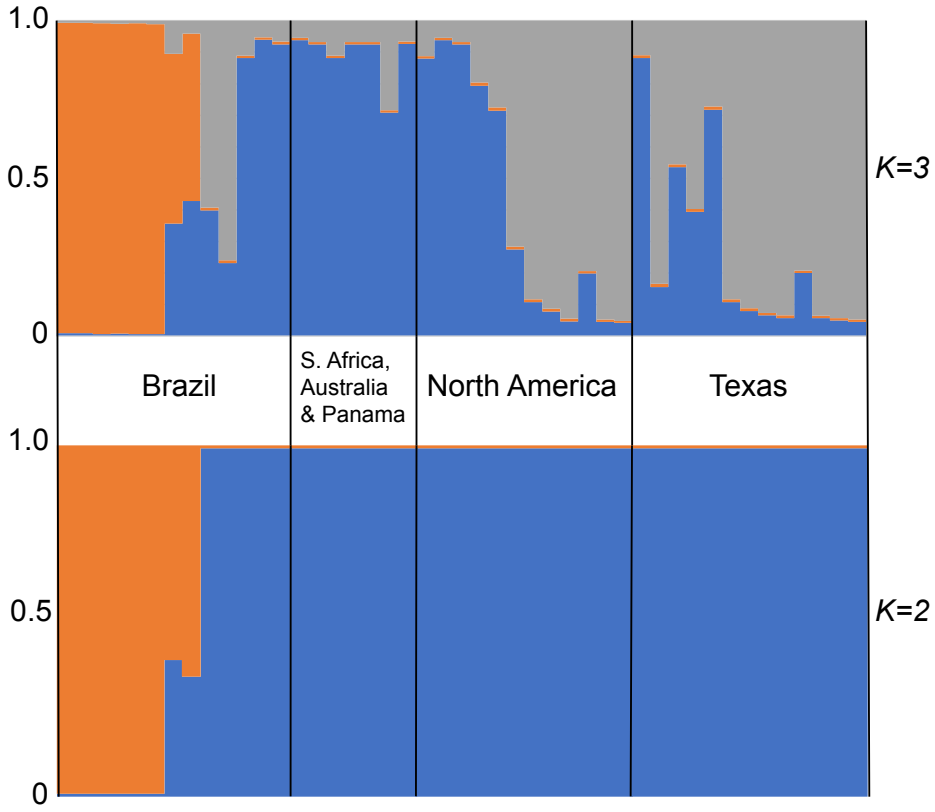

Figure 3B. STRUCTURE Plots for *K*=2 and *K*=3. STRUCTURE results for *K=2* and *K=3* averaged from 5 iterations using CLUMPP. Orange corresponds to *Bd*-GPL, gray corresponds to GPL1, and blue corresponds to GPL2. Assignment probabilities to the clusters are represented on the *y* axis, and each bar represents one of the 45 isolates from a 4 MLST loci dataset, including 19 isolates collected across Texas (from anuran larvae of one genus and two species) for this study and globally distributed strains from Schloegel et al. (2012) and Jenkinson et al. (2016).

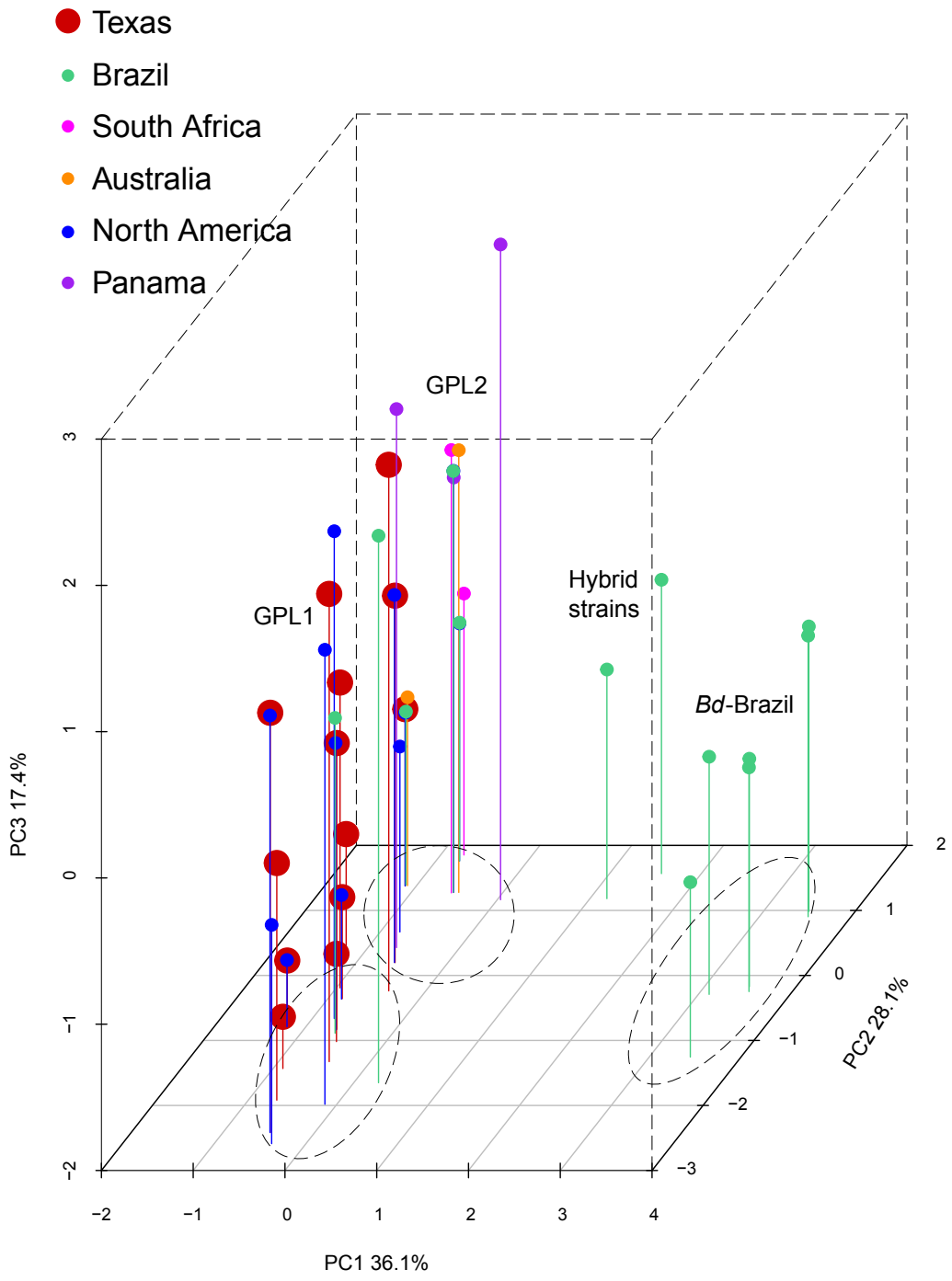

Figure 4. PCA Plot Showing Placement of Texas Isolates in Global Context. A principal components ordination plot of 45 globally distributed *Bd* isolates created using the ADEGENET and ADE4 packages in R and a dataset consisting of 4 MLST loci. Lineages are demarcated with outlines. PC1 explains variation between *Bd*-GPL and *Bd*-Brazil, while PC2 shows differentiation between GPL1 and GPL2.

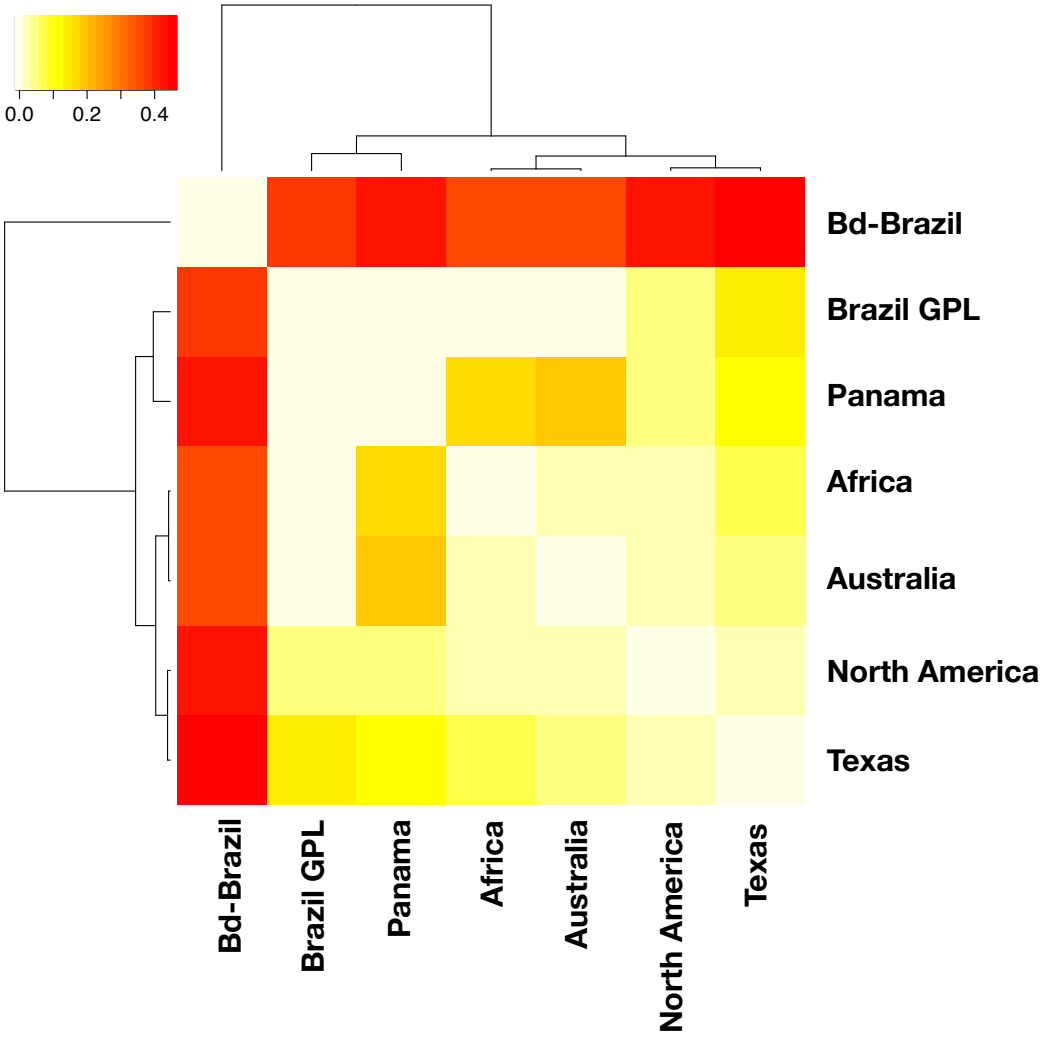

Figure 5. Pairwise F_{ST} Heat Map. A heat map of pairwise F_{ST} values calculated from the dataset of 45 *Bd* isolates, including 19 isolates collected across Texas (from anuran larvae of one genus and two species) for this study and globally distributed strains from Schloegel et al. (2012) and Jenkinson et al. (2016), using the HIERFSTAT package in R.

LITERATURE CITED

Alford RA (2001) Testing the novel pathogen hypothesis. R Speare (Ed.), *Developing management strategies to control amphibian diseases: decreasing the risks due to communicable diseases* (p. 20). School of Public Health and Tropical Medicine, James Cook University, Townsville.

Barr DJS (2001) Chytridiomycota. D McLaughlin, JW Spatafora (Eds.), *Systematics and Evolution* (pp. 93-112). Springer Berlin Heidelberg.

Becker CG, Rodriguez D, Longo AV, Toledo LF, Lambertini C, Leite DS, Haddad CFB, Zamudio KR (2016) Deforestation, host community structure, and amphibian disease risk. *Basic and Applied Ecology,* **17**, 72-80.

Bataille A, Fong JJ, Cha M, Wogan GO, Baek HJ, Lee H, Min M, Waldman B. (2013) Genetic evidence for a high diversity and wide distribution of endemic strains of the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in wild Asian amphibians. *Molecular Ecology,* **22**, 4196-4209.

Bendik N (2017) Demographics, reproduction, growth, and abundance of Jollyville Plateau salamanders (*Eurycea tonkawae*). *Ecology and Evolution*, 1-14.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips, KR, Marantelli G, Parkes H (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America,* **95**, 9031-9036.

Bosch J, Martínez-Solano I, García-París M (2001) Evidence of a chytrid fungus infection involved in the decline of the common midwife toad *(Alytes obstetricans)* in protected areas of central Spain. *Biological Conservation*, **97**, 331–337.

Bowles BD, Sanders MS, Hansen RS (2006) Ecology of the Jollyville Plateau salamander (*Eurycea tonkawae*: Plethodontidae) with an assessment of the potential effects of urbanization. *Hydrobiologia*, **553**, 111-120.

Boyle DG, Hyatt AD, Daszak P, Berger L, Longcore JE, Porter D, Hengstberger SG, Olsen V (2003) Cryo-archiving of *Batrachochytrium dendrobatidis* and other Chytridiomycetes. *Diseases of Aquatic Organisms*, **56**, 59-64.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayres EW (2016) GenBank. *Nucleic Acids Research*, **44**, D67-72.

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science,* **287**, 443-449.

Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. *Diversity and Distributions*, **9**, 141-150.

de Meeûs T, Goudet J (2007) A step-by-step tutorial to use HierFstat to analyse populations hierarchically structured at multiple levels. *Infection, Genetics and Evolution*, **7**, 731-735.

Dray S, Dufour A-B (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1–20.

Earl DA, von Holdt B (2012) Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359-61.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, Bosch J, Cunningham AA, Weldon C, du Preez LH, Anderson L, Pond SLK, Shahar-Golan R, Henk DA, Fisher MC (2011) Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proceedings of the National Academy of Sciences of the United States of America,* **108**, 18732-18736.

Fisher MC, Gow NAR, Gurr SJ (2016) Tackling emerging fungal threats to animal health, food security and ecosystem resilience. *Philosophical Transactions of the Royal Society B,* **371**, 20160332.

Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature,* **484**, 186-194.

Fredericks DN, Relman DA (1996) Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews*, **9**, 18-33.

Gaertner JP, Forstner MR, O'Donnell L, Hahn D (2009) Detection of Batrachochytrium dendrobatidis in endemic salamander species from central Texas. *Ecohealth,* **6**, 20-26.

Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, Kobayashi A, Inaba S, Mizutani T, Hyatt AD (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. *Molecular Ecology,* **18**, 4757-4774.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754-755.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801-1806.

James TY, Litvintseva, AP, Vilgalys R, Morgan JA, Taylor JW, Fisher MC, Berger L, Weldon C, du Preez L, Longcore JE (2009) Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathogens,* **5**, e1000458.

James TY, Toledo LF, Rodder D, da Silva Leite D, Belasen AM, Betancourt-Roman CM, Jenkinson TS, Soto-Azat C, Lambertini C, Longo AV, Ruggeri J, Collins JP, Burrowes PA, Lips KR, Zamudio KR, Longcore JE (2015) Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecology and Evolution,* **5**, 4079-4097.

Jenkinson TS, Betancourt Roman CM, Lambertini C, Valencia-Aguilar A, Rodriguez D, Nunes-de-Almeida CH, Ruggeri J, Belasen AM, da Silva Leite D, Zamudio KR, Longcore JE, Toledo FL, James TY (2016) Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. *Molecular Ecology,* **25**, 2978-2996.

Jombart T (2008) Adegenet: an R package for the multivariate analysis of genetic markers. *Bioinformatics*, **24**, 1403–1405.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647-1649.

Laurance WF, McDonald KR, and Speare R (1996) Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology*, **10**, 406–413.

Letcher PM, Powell MJ (2002) A taxonomic summary of Chytriomyces (Chytridiomycota). *Mycotaxon*, **84**, 447-488.

Letcher PM, Powell MJ, Churchill PF, Chambers JG (2006) Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). *Mycological Research,* **110**, 898-915.

Lips KR (2016) Overview of chytrid emergence and impacts on amphibians. *Philosophical Transactions of the Royal Society B,* **371**, 20150465.

Lips KR, Diffendorfer J, Mendelson JR, Sears MW (2008) Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology,* **6**, e72.

Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, Carey C, Livo L, Pessier AP, Collins JP (2006) Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 3165-3170.

Lips KR, Mendelson III JR, Muñoz-Alonso A, Canseco-Márquez L, Mulcahy DG (2004) Amphibian population declines in montane southern Mexico: resurveys of historical localities. *Biological Conservation,* **119**, 555-564.

Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia,* **91**, 219-227.

Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F (2013) *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences*, **110**, 15325-15329.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE 2010)*, 1-8.

Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, Longcore JE (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology,* **12**, 395-403.

Morgan JA, Vredenburg VT, Rachowicz LJ, Knapp RA, Stice MJ, Tunstall T, Bingham RE, Parker JM, Longcore JE, Moritz C, Briggs CJ, Taylor JW (2007) Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences of the United States of America,* **104**, 13845-13850.

Nielsen-Gammon JW (2011) The changing climate of Texas. J Schmandt, GR North, J Clarkson (Eds.), *The Impact of Global Warming in Texas*, 2nd edn. University of Texas Press, Austin.

Paradis E (2010) PEGAS: an R package for population genetics with an integrated–modular approach. *Bioinformatics*, **26**, 419-420.

Phillips BL, Puschendorf R (2013) Do pathogens become more virulent as they spread? Evidence from the amphibian declines in Central America. *Proceedings of the Royal Society B*, **280**, 20131290.

Pierce BA, Christiansen JL, Ritzer AL, Jones TA (2010) Ecology of Georgetown salamanders (*Eurycea naufragia*) within the flow of a spring. *The Southwestern Naturalist*, **55**, 291-297.

Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia,* **96**, 9-15.

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MP, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, Sanchez-Azofeifa GA, Still CJ, Young BE (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature,* **439**, 161-167.

Powell MJ, Letcher PM, Chambers JG, Roychoudhury S (2015) A new genus and family for the misclassified chytrid, *Rhizophlyctis harderi*. *Mycologia*, **107**, 419-31.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics,* **155**, 945–959.

Rachowicz LJ, Hero JM, Alford RA, Taylor JW, Morgan JAT, Vredenburg VT, Collins JP, Briggs CJ (2005) The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. *Conservation Biology,* **19**, 1441-1448.

Rodriguez D, Becker CG, Pupin NC, Haddad CF, Zamudio KR (2014) Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. *Molecular Ecology,* **23**, 774-787.

Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, Eastman JM, Richards-Hrdlicka K, Joneson S, Jenkinson TS, Longcore JE, Olea GP, Toledo LF, Arellano ML, Medina EM, Restrepo S, Flechas SV, Berger L, Briggs CJ, Stajich JE (2013) Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proceedings of the National Academy of Sciences of the United States of America,* **110**, 9385-9390.

Saenz D, Kavanagh BT, Kwiatkowski MA (2010) *Batrachochytrium dendrobatidis* detected in amphibians from National Forests in Eastern Texas, USA. *Herpetological Review,* **41**, 47.

Savage AE, Zamudio KR (2011) MHC genotypes associate with resistance to a frog-killing fungus. *Proceedings of the National Academy of Sciences*, **108**, 16705-16710.

Savage AE, Zamudio KR (2016). Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. *Proceedings of the Royal Society B,* **283**, 20153115.

Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira CA, Lee M, Zhao S, Wangen C, Ferreira CM, Hipolito M, Davies AJ, Cuomo CA, Daszak P, James TY (2012) Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. *Molecular Ecology,* **21**, 5162-5177.

Sparrow FK (1960) *Aquatic Phycomycetes*, 2nd ed. University of Michigan Press, Ann Arbor.

Stevenson LA, Alford RA, Bell SC, Roznik EA, Berger L, Pike DA (2013) Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *PloS One,* **8**, e73830.

Tipton BL, Hibbitts TL, Hibbitts TD, Hibbitts TJ, LaDuc TJ (2012) *Texas Amphibians: A Field Guide*, 1st edn. University of Texas Press, Austin.

Vander Wal E, Garant D, Calme S, Chapman CA, Festa-Bianchet M, Millien V, Rioux-Paquette S, Pelletier F (2014) Applying evolutionary concepts to wildlife disease ecology and management. *Evolutionary Applications,* **7**, 856-868.

Velo-Anton G, Rodriguez D, Savage AE, Parra-Olea G, Lips KR, Zamudio KR (2011) Amphibian-killing fungus loses genetic diversity as it spreads across the New World. *Biological Conservation,* **146**, 213-218.

Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ, Wake DB (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proceedings of the National Academy of Sciences of the United States of America,* **107**, 9689-9694.

Weldon C, du Preez LH, Hyatt AD, Muller R, Speare R (2004) Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases,* **10**, 2100-2105.

Woodhams DC, Ardipradja K, Alford RA, Marantelli G, Reinert LK, Rollins-Smith LA (2007) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. *Animal Conservation*, **10**, 409-417.